Parainfluenza Virus Infection

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Abstract

Human parainfluenza viruses (HPIVs) are single-stranded, enveloped RNA viruses of the Paramyoviridaie family. There are four serotypes which cause respiratory illnesses in children and adults. HPIVs bind and replicate in the ciliated epithelial cells of the upper and lower respiratory tract and the extent of the infection correlates with the location involved. Seasonal HPIV epidemics result in a significant burden of disease in children and account for 40% of pediatric hospitalizations for lower respiratory tract illnesses (LRTIs) and 75% of croup cases. Parainfluenza viruses are associated with a wide spectrum of illnesses which include otitis media, pharyngitis, conjunctivitis, croup, tracheobronchitis, and pneumonia. Uncommon respiratory manifestations include apnea, bradycardia, parotitis, and respiratory distress syndrome and rarely disseminated infection. Immunity resulting from disease in childhood is incomplete and reinfection with HPIV accounts for 15% of respiratory illnesses in adults. Severe disease and fatal pneumonia may occur in elderly and immunocompromised adults. HPIV pneumonia in recipients of hematopoietic stem cell transplant (HSCT) is associated with 50% acute mortality and 75% mortality at 6 months. Though sensitive molecular diagnostics are available to rapidly diagnose HPIV infection, effective antiviral therapies are not available. Currently, treatment for HPIV infection is supportive with the exception of croup where the use of corticosteroids has been found to be beneficial. Several novel drugs including DAS181 appear promising in efforts to treat severe disease in immunocompromised patients, and vaccines to decrease the burden of disease in young children are in development.

Keywords
- parainfluenza virus infection

Human parainfluenza viruses (HPIVs) are an important cause of respiratory illness in children and adults with a wide range of clinical manifestations including colds, croup, bronchiolitis, and pneumonia. Seasonal HPIV virus epidemics result in a significant burden of disease in children and account for 40% of pediatric hospitalizations for lower respiratory tract illnesses (LRTIs) and 75% of croup cases.[1–6] Immunity resulting from disease in childhood is incomplete and reinfection occurs throughout adult life, although symptoms are typically mild and self-limited. However, in immunocompromised or elderly adults, infection may progress to the lower respiratory tract and cause severe and life-threatening pneumonia.[7–10] Though sensitive molecular diagnostics are now available to rapidly diagnosis parainfluenza infection, effective therapies are still needed and treatment remains supportive. In this review, we will summarize the epidemiology, clinical manifestations, diagnostic methods, and treatment options for HPIV infection.

Virology

Parainfluenza viruses are single-stranded, enveloped RNA viruses of the Paramyoviridaie family. There are four major serotypes of HPIV, noted as serotypes 1 to 4 with human

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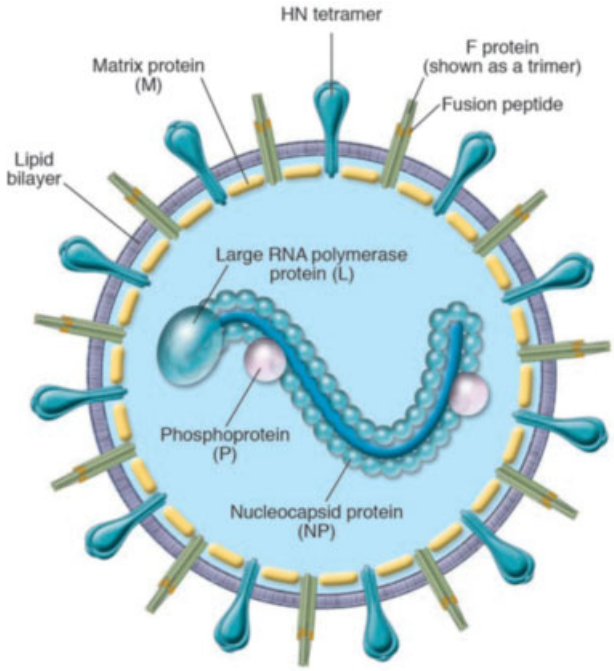

Fig. 1 Structure of human parainfluenza virus serotypes 1 to 4. Parainfluenza viruses are single-stranded, enveloped RNA viruses and virions are pleomorphic, ranging in diameter from 150 to 200 µm. The RNA encodes six essential proteins in a conserved order: the nucleocapsid protein (NP), the phosphoprotien (P), the matrix protein (M), the fusion glycoprotein (F), the hemagglutinin neuraminidase glycoprotein (HN), and RNA polymerase (L). (Reproduced with permission from Moscona A. Entry of parainfluenza virus into cells as a target for interrupting childhood respiratory disease. J Clin Invest 2005;115:1688–1698.[13])

HPIV4 subdivided into two genera (HPIV4a and HPIV4b).[11] HPIV1 and HPIV3 are members of the genus *Respirovirus* and the genus *Rubulavirus* includes HPIV2 and HPIV4. Parainfluenza virions are pleomorphic, ranging in diameter from 150 to 200 µm (►**Fig. 1**).[12,13] They contain a single, negative-sense RNA strand which encodes six essential proteins in a conserved order: the nucleocapsid protein (NP), the phosphoprotien (P), the matrix protein (M), the fusion glycoprotein (F), the hemagglutinin neuraminidase (HN) glycoprotein, and the RNA polymerase (L).

The HN and fusion glycoproteins are surface proteins which mediate attachment to the sialic acid residues on the surface of host epithelial cells (HN) and fusion of the viral envelope with the host cell membrane (F), respectively (►**Fig. 2**).[13,14] The HN protein also facilitates release of new virions from the cell by cleaving the sialic acid residue.[15,16] These two proteins are the major targets for neutralizing antibodies. The matrix protein coats the inner surface of the envelope. The NP protein binds and coats the viral RNA, creating a template for the RNA-dependent RNA polymerase, consisting of the P and L proteins, to facilitate transcription. The P gene also encodes additional proteins which vary among the four serotypes and are not essential for virus replication.[17] HPIV1 and HPIV3 RNAs encode short C proteins and HPIV2 RNA encodes a V protein both of which suppress the host immune response by decreasing type 1 interferon activity. A third nonessential protein, D protein, is expressed by HPIV3, though the relevance and function of this protein remains unclear. Replication occurs in the cytoplasm of the host cell and once produced the negative-sense RNA strands are packaged and exported as new virions.

The hemagglutinin neuramindase proteins are more stable for parainfluenza viruses compared with those of influenza A viruses. However, antigenic differences have been noted over time, producing strains serologically and genetically different from earlier isolates and impeding vaccine development.[11,18–20]

Pathogenesis

Parainfluenza viruses bind and replicate in the ciliated epithelial cells of the upper and lower respiratory tract.[13,21] Infection begins in the nose and oropharynx and then spreads to the lower airways with peak replication 2 to 5 days after initial infection.[22] The extent of infection correlates with location, that is, cold symptoms are associated with infection in the upper airways, infection of the larynx and trachea results in croup and bronchiolitis, and pneumonia occurs with replication in the distal airways.[23,24] Once epithelial cells of the small airways become infected, inflammatory infiltrates develop and the host immune response is thought to contribute to disease pathogenesis.[25,26] The classic signs of croup include hoarseness, cough, and stridor which are due to obstruction from inflammation of the subglottic region of the trachea (►**Fig. 3**).[27] This area is less distensible than other parts of the trachea because it is encircled by the cricoid cartilage. The impeded airflow produces the high pitched inspiratory vibrations known as stridor and increased work of breathing due to this obstruction may lead to fatigue and hypoxia and eventually respiratory failure in severe cases. Adult illness is generally mild, although airway hyper responsiveness may occur in persons with asthma due to release of cytokines and chemokines.[28]

Immunology

Host defense against HPIV is mediated by both humoral and cellular immunity.[11] Serum antibodies directed against the two surface glycoproteins, F and HN, are neutralizing and protective against challenge.[29,30] Secretory immunoglobulin A (IgA) also develops after natural infection and has been shown to neutralize virus and ameliorate disease.[11] Neutralizing antibody appears to be serotype specific with little cross protection afforded by antibodies between HPIV serotypes 1 to 4.[31] Cytotoxic T lymphocyte responses are important for clearance of virus, and T cell epitopes have been demonstrated on the HN, P, and NP proteins of HPIV.[11,32–34] Repeated infections are often needed to fully protect a child's lower respiratory tract from HPIV infection and eventual protection may be a combination of high levels of neutralizing antibody and cellular immunity.[11,35] Immunity to HPIV is incomplete and reinfections with any of the HPIV serotypes can occur throughout life.

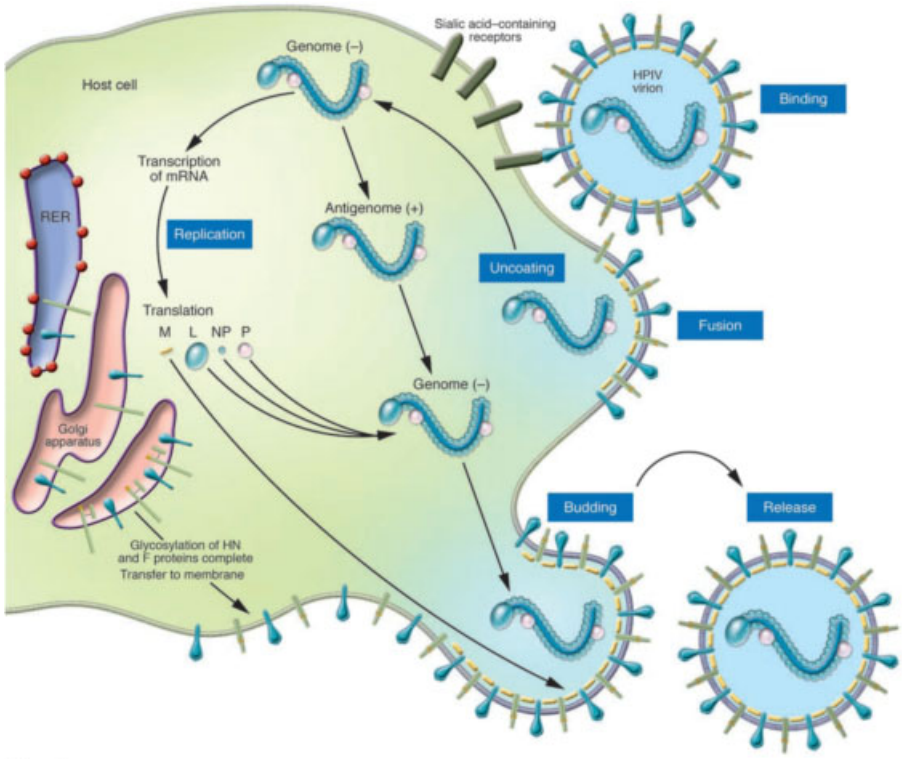

Fig. 2 Cycle of attachment, fusion, and replication for parainfluenza viruses. The HN glycoproteins attach to sialic acid residues on the surface of host epithelial cells and fusion glycoprotein mediate fusion of the viral envelope with the host cell membrane. After attachment, the genetic material is uncoated and replication occurs in the cytoplasm of host cells. The NP protein binds the viral RNA, creating a template for the RNA-dependent RNA polymerase, consisting of the P and L proteins. Once replication is completed, HN and F proteins are transferred to the host cell membrane which forms the envelope for new virons which is coated on the inner surface by the matrix protein. The HN protein then facilitates budding and release of new virions from the cell by cleaving the sialic acid residues. (Reproduced with permission from Moscona A. Entry of parainfluenza virus into cells as a target for interrupting childhood respiratory disease. J Clin Invest 2005;115:1688–1698.[13])

Epidemiology

HPIVs were first isolated from children with croup in 1955 and were referred to as croup-associated viruses.[36,37] They have been shown to cause upper respiratory tract infection (URTI) in children and adults, and LRTI in children younger than 5 years and elderly or immunocompromised adults, demonstrating a distinctly bimodal pattern of age distribution.[38] Transmission occurs through direct person-to-person contact or from large droplets, and household outbreaks have been well described in the literature, as have outbreaks in nursing home and daycare facilities.[39]

Parainfluenza virus infections occur throughout the world with seasonal variations in serotype-specific rates of infection which is determined by region.[38] Seasonal patterns of infection noted in the northern hemisphere are absent in tropical and subtropical regions with little variation in infection rates throughout the year.[40] In the United States, HPIV1 typically causes biennial outbreaks in odd-numbered years during the fall and may be responsible for 50% of croup cases in the United States during epidemic seasons (►**Fig. 4**).[3,6,11] Epidemics of HPIV2 infections occur annually in the fall and HPIV3, and the most prevalent serotype causes seasonal outbreaks in the spring, usually following influenza epidemics.[38] In years when HPIV1 is not actively circulating, a second smaller HPIV3 epidemic may occur in the fall. In contrast, the epidemiology of the HPIV4 infections has not been well studied with only few reports of small number viruses isolated from children and adults.[41–43] This is due to the fact that illness related to HPIV4 infection is often mild and subclinical and the virus more difficult to detect.[41,43–45]

Other trends in rates and severity of infection with HPIV have been described including reduced risk of severe illness in breast-fed infants and after pneumococcal vaccination. Increased risk of progressive to severe illness is noted in immunocompromised hosts, especially in those with hematologic malignancies, hematopoetic stem cell transplant (HSCT), or solid-organ transplantation. Additionally, socioeconomic factors such as malnutrition, overcrowding, vitamin A deficiency, and environmental smoke or toxins have also been shown to predispose children to HPIV infections.[11,46–49] Finally, gender and ethnicity also appear to play a role, as PIV-associated bronchiolitis reportedly occurs more often in nonwhite males.

Serotype prevalence: HPIV3 is the most commonly isolated serotype in symptomatic disease for both children and adults.[38] In the National Respiratory and Enteric Viruses Surveillance System study conducted from 1990 to 2004, HPIV3 was the most commonly identified serotype (52%), followed by HPIV1 (26%), HPIV2 (12%), and HPIV4 (2%).[38]

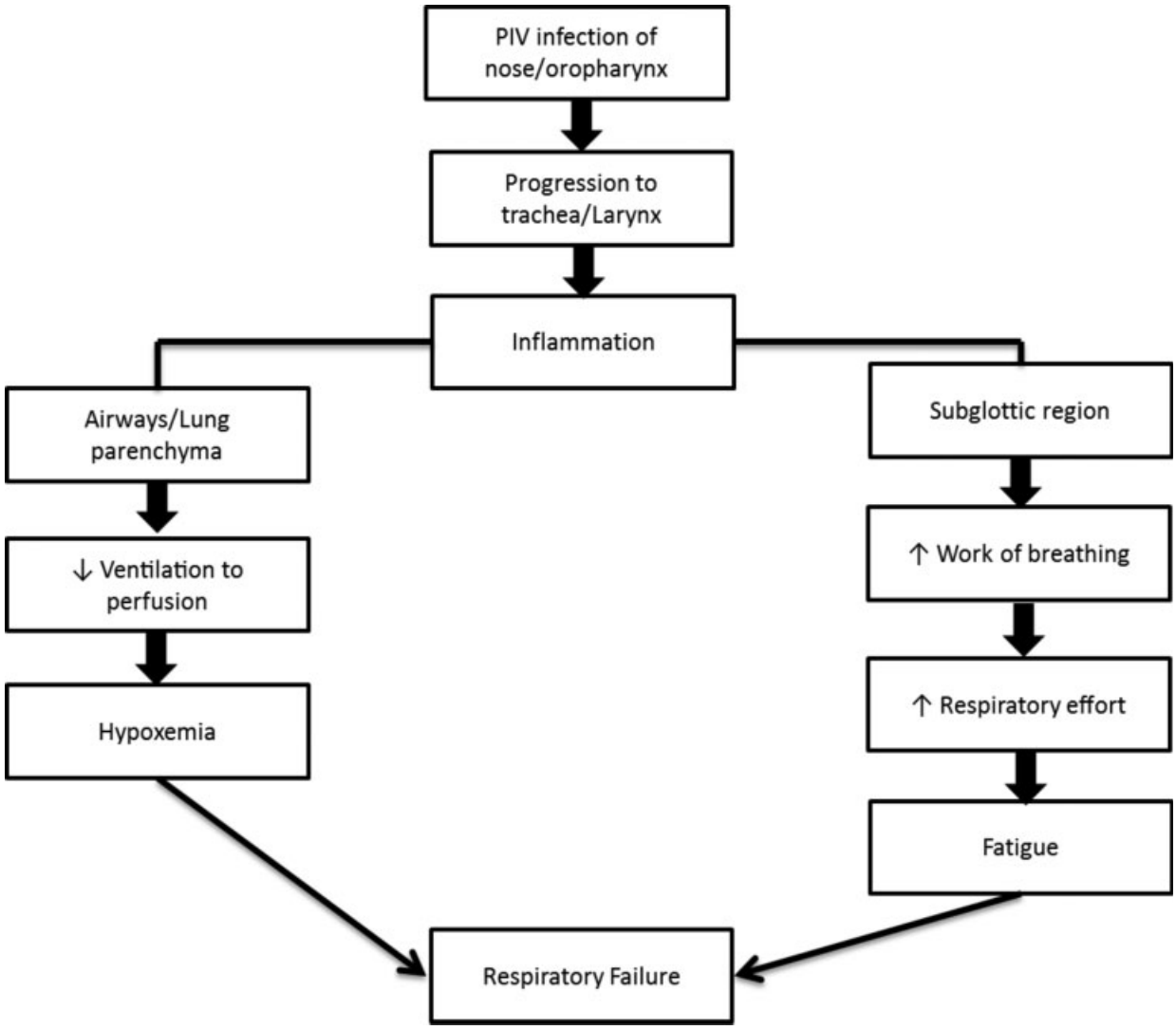

Fig. 3 Pathogenesis and disease progression of HPIV-associated croup. (Adapted from Bower J and McBride JT, Principles and Practices of Infectious Diseases. 8th Edition 2015.[27])

However, during epidemic years, HPIV1 is associated with significant disease burden and hospitalizations in children.[3,4,6,11,50,51] Moreover, several studies have demonstrated the importance of this virus as a cause of yearly hospitalizations in adults and nursing outbreaks associated with bacterial coinfection and fatal pneumonia.[52] Acquisition of HPIV3 usually occurs earliest in life with 50 and 92% of children infected by 1 and 3 years of age, respectively (►**Fig. 5**).[1,5] Primary infection with HPIV1 and HPIV2 occurs later in childhood (age 2–6 years).

Children: There are more than 5 million cases of children with LRTI in the United States each year, and HPIV accounts for 20 to 40% of these illnesses.[1,2,53] Population-based studies estimate 1.9 to 12 per 1,000 children younger than 1 year and 0.5 to 2.0 per 1,000 children aged 1 to 4 years are infected each year with HPIV.[54–56] In outpatient studies, HPIV accounts for approximately 17% of viral respiratory illnesses identified in children (18% of upper respiratory illnesses, >20% of LRTI, and >50% of croup cases).[5,53] The U.S. 2000 Census estimates that rates of medically attended acute respiratory illness, LRTI, and hospitalization in children younger than 5 years associated with HPIV3 infection were 3.2 million, 1.1 million, and 29,000, respectively.[57] Other reports estimate 7,600 to 48,000 pediatric hospitalizations annually in the United States, and 7% of pediatric hospitalizations for febrile or respiratory illnesses in children younger than 5 years are due to HPIV. In composite, pediatric hospitalizations and emergency room visits due to HPIV constitute a cost of more than $200 million annually.[4]

Adults: New molecular viral diagnostics have resulted in greater understanding of the impact of HPIV infections in adult populations and reinfection has been found to be common.[58–62] PIV infections account for 1 to 15% of respiratory illnesses in adults with infrequent reports of pneumonia in young adults and higher risk for severe disease in frail older adults.[38,60] It is estimated that 2 to 11.5% of adult hospitalizations for respiratory illnesses are due to HPIV infection.[59,61,63–65] Furthermore, HPIV is frequently implicated in acute exacerbations of chronic obstructive pulmonary disease with HPIV usually the 2nd or 3rd most commonly

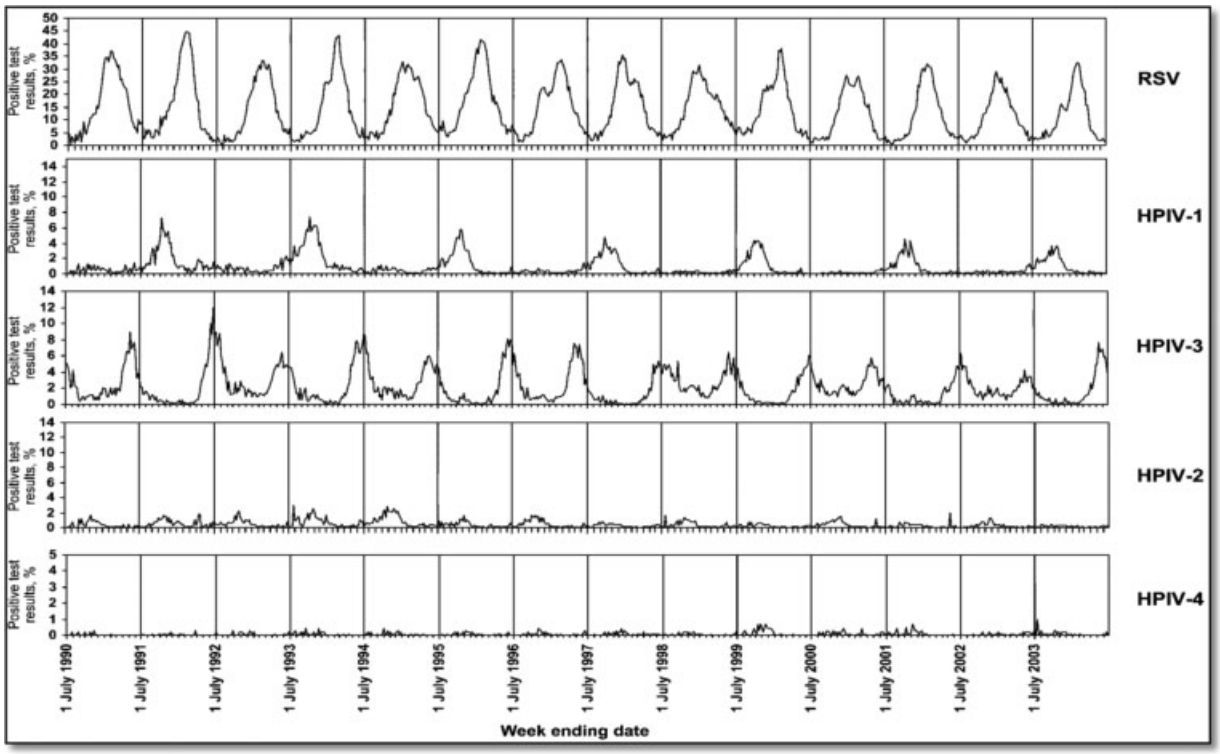

Fig. 4 The percentage of tests positive for human parainfluenza virus (HPIV) serotypes 1, 3, 2, and 4 reported to the National Respiratory and Enteric Viruses Surveillance System (NREVSS), by week, July 1990 to June 2004. (Reproduced from Fry AM, Curns AT, Harbour K, et al. Seasonal trends of human parainfluenza viral infections: United States, 1990–2004. Clin Infect Dis 2006;43:1016-1022.[38])

detected virus (►**Fig. 6**).[66–69] HPIV infection has also been reported in long-term care facilities with prospective studies documenting 4 to 14% of nursing home residents infected annually. Fatal bronchopneumonia is an infrequent but reported outcome in this population and has been associated with HPIV1 outbreaks.[52,60,70–76] During epidemic seasons, HPIV1 and HPIV3 outbreaks have been reported and with very high attack rates.[52,73] One report from an Alabama nursing home described attack rates of 22 and 28% in residents and employees, respectively.[71] In an outbreak at a California state mental hospital, 56% of residents were infected.[71]

Finally, severe LRTI disease and pneumonia have been reported in immunocompromised hosts, particularly patients with hematologic malignancies and HSCT recipients. Reports indicate an incidence of HPIV-associated respiratory illness in

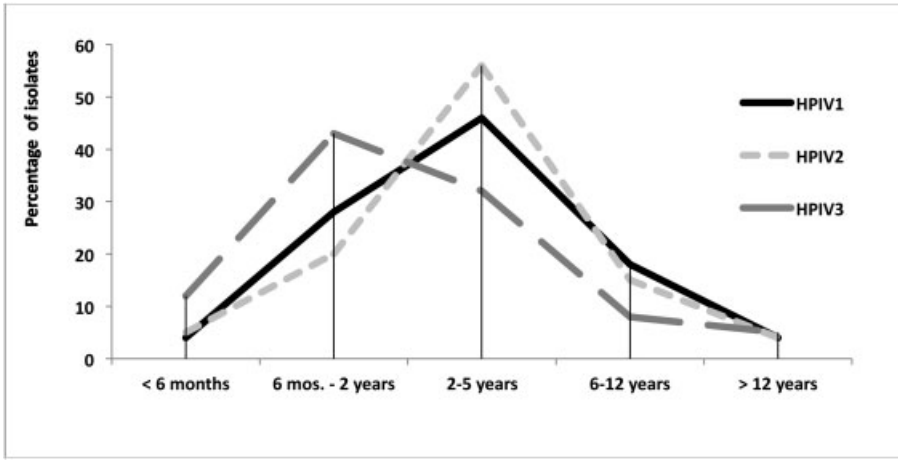

Fig. 5 Age distribution of parainfluenza serotypes 1, 2, and 3 viral infections in outpatient children. The y-axis represents the percentage of children for whom infection with the three parainfluenza virus serotypes was detected per age group. Vertical lines identify serotype with the highest incidence of infection per age group. (Adapted from Knott, AM, Long, CE, et al. Parainfluenza viral infections in pediatric outpatients: seasonal patterns and clinical characteristics. J Pediatr Infect Dis 1994, 13:269–73.[5])

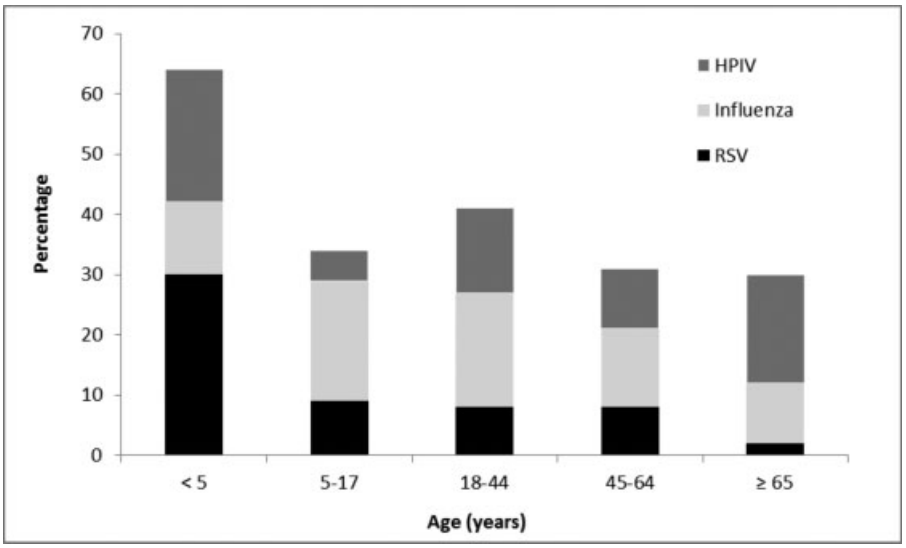

Fig. 6 Comparison of influenza, parainfluenza, and RSV infections in hospitalized patients with chronic underlying conditions. Chronic pulmonary conditions included asthma, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, sarcoidosis, and malignancy or asbestosis. Other chronic conditions included congestive heart failure, metabolic disease (i.e., diabetes), chronic anemia, chronic renal disease, and malignancies or immunocompromising conditions. The percent of viruses is shown distributed by age group. Black, respiratory syncytial virus (RSV); white, influenza; gray, human parainfluenza virus (HPIV). (Adapted from Glezen, WP et al. Impact of respiratory virus infections on persons with chronic underlying conditions. JAMA 2000;283(4):499–505.[67])

the HSCT population of 2 to 7%, with HPIV3 accounting for 90% of infections and nosocomial epidemics reported in bone marrow transplant wards during peak seasons.[7–10] Pneumonia has been reported in 24 to 55% of HSCT patients infected with HPIV and is associated with up to 50% acute mortality rate and 75% mortality rate at 6 months.[7,77–79] Though not as frequently reported in patients with solid-organ transplants, severe disease has also been documented in this population. HPIV infection may result in severe complications for lung transplant recipients including bronchiolitis obliterans, reduced lung function, and allograft rejection.[80–82]

Clinical Manifestations

Parainfluenza viruses are associated with both upper and lower respiratory tract disease in children and adults, and the spectrum of illness typically includes otitis media, pharyngitis, conjunctivitis, croup, tracheobronchitis, and pneumonia. Uncommon respiratory manifestations include apnea, bradycardia, parotitis, and respiratory distress syndrome. Although HPIV primarily infects respiratory tissues, disseminated infection has been described as having a variety of illnesses affecting other organ systems, including neurologic, renal, and rheumatologic diseases.

Pediatric disease: In children, 40 to 60% of HPIV infections result in URTIs (colds and pharyngitis) and approximately 30 to 50% of these illnesses are complicated by otitis media.[5,53,83] URTI is the predominant presentation for all serotypes and less than 20% of HPIV infections result in lower respiratory tract disease other than croup.

HPIV1 and HPIV2 are the leading causes of croup, accounting for 60 to 75% of croup illnesses and contributing 27,000 to 66,000 pediatric hospitalizations yearly (►**Fig. 7**).[3–6,53] Croup caused by HPIV2 is generally milder but can result in significant airway compromise and hospitalization. In contrast, HPIV3 infection is more commonly associated with LRTI than other serotypes causing bronchiolitis and pneumonia in neonates and infants with illness that is clinically indistinguishable from RSV infection.[84] Illness related to HPIV4 infection appears to be most commonly associated with URTI symptoms.

Croup (acute laryngotracheitis and acute laryngotracheobronchitis): The characteristic anatomic finding of croup is inflammation of the larynx and trachea otherwise known as

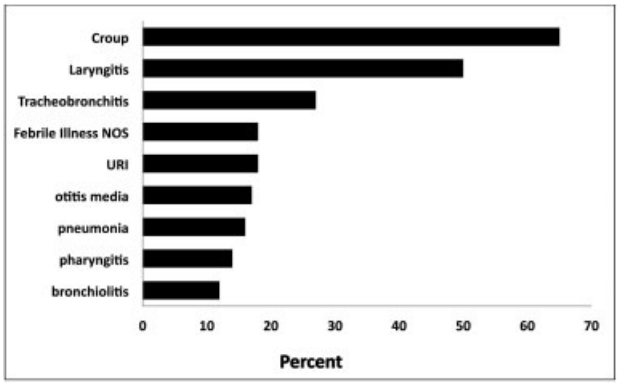

Fig. 7 Proportion of clinical syndromes associated with parainfluenza infection in children found in a survey of respiratory illnesses in children in an outpatient setting with a known viral etiology. Upper respiratory tract infection (URTI) indicates colds with or without fever. NOS indicates an undifferentiated febrile illness. (Adapted from Knott, AM, Long, CE, et al. Parainfluenza viral infections in pediatric outpatients: seasonal patterns and clinical characteristics. J Pediatr Infect Dis 1994, 13:269–273.[5])

laryngotracheitis. When inflammation extends into the bronchi (laryngotracheobronchitis), lower airway signs such as wheezing and air trapping also occur. These two terms are used interchangeably to represent croup disease and are often clinically indistinct. Extension of disease into the lower airways increases the risk for bacterial infection with typical respiratory pathogens (*Staphylococcus aureus*, *Strepotococcus pneumonia*, *Morxaxella catarrhalis*, and *Haemophilus influenza*) which may present as mucopurulent bacterial tracheitis or pneumonia.

Croup incidence peaks at 1 to 2 years of age and reports indicate that disease occurs more frequently in male children who have a 1.43 higher risk of having croup than their female counterparts, with the highest risk to boys noted between the ages of 6 and 12 months.[3] Epidemiological studies in Milwaukee have also reported increased risk for disease in white children compared with black children. Approximately 8 to 15% of children with croup will require hospitalization and 1 to 3% will require intubation.[55,85–88]

Children with croup typically present initially with fever, hoarseness, and rhinorrhea with or without pharyngitis which progresses in 12 to 48 hours to the characteristic hoarse "barking" cough. Laryngeal obstruction follows in moderate to severe cases, manifested by inspiratory stridor. The characteristic "steeple sign" can be seen on chest or neck radiograph (►**Fig. 8**). As airway obstruction progresses, chest wall retractions are generally accompanied by worsening agitation and increased inspiratory effort which paradoxically exacerbates the obstructive process. Hypoxia, cyanosis, and respiratory fatigue may develop, requiring intubation which can rarely be fatal (<0.5% of intubated patients).[89] The diagnosis of croup is made clinically and severity measured by five clinical factors known as the Westley scale: mental status, the presence of absence of pallor or cyanosis, the presence of absence of inspiratory stridor at rest, the degree of chest wall retractions, and the amount of air entry (►**Table 1**).[90] Mild croup is characterized by the absence of stridor at rest and can often be managed symptomatically at home. In contrast, children with moderate to severe croup will present with inspiratory stridor at rest accompanied by variable degrees of respiratory compromise and should be evaluated in an acute care setting. Symptoms of croup typically resolve in 1 to 3 days with appropriate therapy (see section "Treatment") but may persist for up to 7 days. Worsening symptoms after a period of improvement should prompt evaluation for bacterial complications.[91]

Bronchiolitis: Bronchiolitis results from infection of the small airways (bronchioles) of infants and young children and 90% of illnesses are due to viral infection, mostly often with respiratory syncytial virus (RSV).[92] However, all four HPIV serotypes can cause this syndrome and 10 to 20% of confirmed viral bronchiolitis infections due to HPIV1 and HPIV3.[47,56] Typical illness begins with a prodrome of fever and nasal congestion 1 to 3 days prior to the onset of lower respiratory signs and symptoms (cough, expiratory wheezing, tachypnea, rales, and chest wall retractions). Symptoms peak at 5 to 7 days and 90% of children without underlying cardiopulmonary disease recover from bronchiolitis within 21 days, with a median duration of symptoms of 8 to 15 days.[91,93] However, 10% of children will have persistent symptoms of cough and wheezing for 1 to 2 weeks longer and disease may be more severe and the course prolonged in premature or young infants and those with comorbid

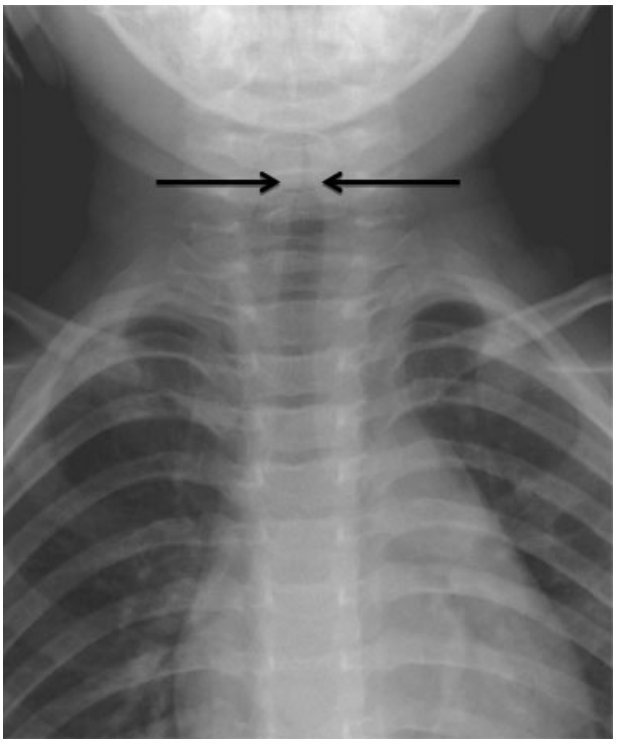

Fig. 8 Anterior/Posterior chest radiograph demonstrating tapering of the upper trachea or "steeple" sign seen in parainfluenza-associated croup infections. Arrows indicate area of obstruction resulting from inflammation of the subglottic region of the trachea. (Reproduced with permission from Huang,Chun-Chao and Shih, Shin-Lin. Steeple Sign of Croup. N Engl J Med 2012; 367:6.[207])

Table 1 Westley croup severity score

Clinical feature	Score
Mental status	0 = Normal 5 = Altered
Cyanosis	0 = None 4 = With agitation 5 = At rest
Stridor	0 = None 1 = With agitation 2 = At rest
Air entry	0 = Normal 1 = Decreased 2 = Markedly decreased
Retractions	0 = None 1 = Mild 2 = Moderate 3 = Severe

Notes: ≤ 2, mild; 3–7, moderate; 8–11, severe; ≥12, impending respiratory failure.

conditions (i.e., bronchopulmonary dysplasia, congenital heart defects, or immunosuppression). Children with severe disease are at risk for complications including apnea and respiratory failure requiring mechanical ventilation.[92]

Pneumonia: Pneumonia in children classically presents with fever, cough, and rales with infiltrates or consolidation on chest radiographs. Though all four parainfluenza serotypes have been associated with pneumonia in children, HPIV1 and HPIV3 are most often implicated, accounting for 1 to 6% and 2 to 12% of HPIV-related hospitalizations, respectively.[11,47,56] The clinical syndrome of HPIV pneumonia in children is not distinctive. Pneumonic infiltrates are usually described as bilateral interstitial infiltrates, though alveolar infiltrates can be seen.[94] Although data are limited, bacterial complications of HPIV in a normal child appear uncommon (<15%) and may be associated with severe and necrotizing pneumonia (►**Fig. 9**).[94,95] Treatment is supportive and expectation of recovery is similar to that for bronchiolitis disease.

Tracheobronchitis: Tracheobronchitis is a term used to describe disease that does not fit well into other classical syndromes but generally involves inflammation of the large airways, that is, trachea and bronchi, in the absence of symptoms of croup and radiologic findings of pneumonia. In addition to upper respiratory tract symptoms and fever, patients may have a productive cough, wheezing, and rhonchi. It is generally a clinical syndrome seen in older children and associated with HPIV viral infection in a fourth of all cases.[47] HPIV3 is most commonly associated with this syndrome, though some reports have noted a pattern of large airway disease with HPIV4 infections as well.[96]

Adult disease in immunocompetent hosts: HPIV infections in healthy adults are generally mild, self-limited URTIs with typical cold symptoms (rhinorrhea, cough, and sore throat) with or without fever.[56,58,60,61,73,97,98] Otitis media and sinusitis may occasionally complicate adult infection.[99] HPIV may also cause pneumonia, particularly in frail older adults.[59,64,66,100–102] Signs and symptoms of HPIV infection are indistinguishable from other viruses such as influenza and RSV and may be overshadowed by findings associated with exacerbations of chronic medical conditions such as chronic obstructive pulmonary disease (COPD) and congestive heart failure.[103] Radiographic findings consist of patchy unilateral or bilateral infiltrates, though 50% of HPIV-associated pneumonia may be complicated by bacterial infection.[104] HPIV infection is also increasingly recognized as a cause of acute exacerbation of COPD and asthma.[68,69,105–110] Infection may result in severe illness, deterioration of lung function, and prolonged hospitalization requiring ICU care and mechanical ventilation.

Immunocompromised hosts: Parainfluenza viruses cause severe infections in immunocompromised children and adults and have been associated with significant morbidity and mortality. Recent studies in HSCT and leukemic patients estimated the incidence of symptomatic HPIV infections to be between 2 and 7%.[7,9,48,78,111] The majority of these illnesses are community acquired (80%), though outbreaks in HSCT wards have been described with 90% due HPIV3.[77,78]

At presentation, most patients (70%) will have upper respiratory tract symptoms of cough, rhinorrhea, and sore throat with or without fever.[112] The presence of URTI symptoms may be the best clue that illness is due to a respiratory virus and distinguishes infection from the myriad of other infectious agents affecting this population.[111] Progression to lower respiratory tract involvement occurs in 43% of HSCT recipients and 55% of leukemia patients and results in high rates of mortality (37–50%).[7,9,77,78,111] Mortality hazard ratio for HPIV-related URTI is 1.3 compared with 3.4 for LRTI.[78] In a recent retrospective report, 30% of the 80 leukemic and 120 HSCT patients at a large cancer center diagnosed with HPIV infections presented with pneumonia. Of those with URTI symptoms initially, 61% of leukemic patients and 39% of HSCT subjects progressed to pneumonia during their illness.[112] In children, similar rates of progression to LRTI disease have been reported with one retrospective study finding that 47% of all viral infections in 274 pediatric HSCT recipients were caused by HPIV viruses and of

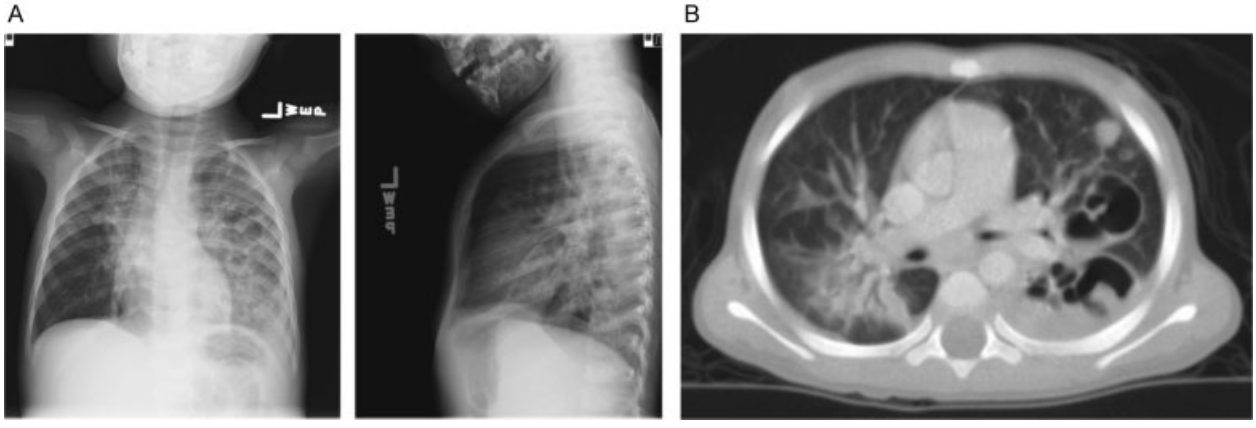

Fig. 9 Chest radiograph and chest computed tomography (CT) of a 5-year-old child with HPIV3-associated necrotizing community-acquired pneumonia. Viral PCR of a nasopharyngeal sample was positive for HPIV3. Blood culture was positive for methicillin-resistant *Staphylococcus aureus*. Chest radiograph (**A**) reveals bilateral, multifocal infiltrates and a small left pleural effusion. (**B**) CT confirms dense consolidation in the left lower lobe, right upper, and right lower lobes; numerous satellite nodules in the left lower and upper lobe bronchiectasis; and multifocal cystic lesions bilaterally. (Reproduced with permission from Derek J. Williams, and Samir S. Shah J Pediatr Infect Dis 2012;1:1–5.[94])

those, 41% had pneumonia.[113] Similarly, risk for severe disease is also increased in children undergoing chemotherapy, with the highest risk noted in children younger than 2 years with hematologic malignancies.[114] Other factors associated with progression to lower respiratory tract disease include dose-dependent treatment with steroids, allogenic SCT (vs. autologous), time from transplantation (majority of cases in HSCT patients occur in <100 days posttransplantation), presence of lymphopenia, and pediatric age group.[7,78,114]

HSCT and leukemic patients with HPIV pneumonia or pneumonitis will commonly present with fever (77–89%), cough, (62–85%), dyspnea (43–82%), sputum production (67%), nasal congestion or rhinorrhea (52%), and sore throat (30%).[7,77] Chest imaging may reveal a wide variety of findings, including unilateral or bilateral infiltrates on chest radiograph and CT scan findings of interstitial infiltrates, groundglass opacities, peribronchial nodules, and/or airspaces consolidations (►**Fig. 10**).[115,116] Diagnosis is made by identifying HPIV in respiratory secretions. Appropriate workup should be obtained to rule out bacterial and fungal copathogens because they frequently (26–61%) complicate HPIV infections and contribute significantly to mortality in this population.[7,77–79] In one report from a single center, 53% of patients with HPIV3 pneumonia were found to be coinfected with other pathogens and mortality in this group was 35% at 30 days and 75% at 180 days.[78] In another study reporting 37% mortality associated with PIV pneumonia, 7 of the 10 patients who died had concurrent infections with other respiratory pathogens.[7] The most frequently isolated organisms are *Aspergillus fumigatus*, often associated with fatal pneumonia, cytomegalovirus, and *Pseudomonas aeruginosa* with a variety of other gram-negative bacterial pathogens reported as well.[7,77–79]

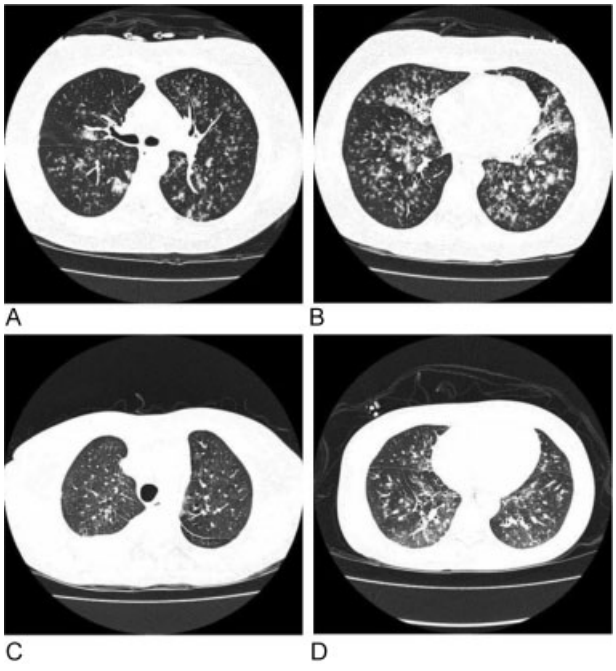

Fig. 10 Patterns found on high-resolution chest computed tomography (CT) in hematopoietic stem cell transplant recipients with HPIV3 pneumonia. (**A**) Peribronchial nodules. (**B**) Nodules and associated consolidation. (**C**) Very small peribronchial nodules in the left upper lobe. (**D**) Multiple small peribronchial nodules and ground-glass consolidation. (Reproduced with permission from Ferguson PE, et al. Clin Infect Dis 2009;48:905–909.[116])

Limited studies have demonstrated a more modest impact of HPIV infections in solid-organ transplants recipients. Anecdotal reports have described isolated cases of HPIV infection associated with acute rejection in renal and liver transplant patients.[117,118] However, the majority of studies have assessed disease associated with HPIV infections in lung transplant patients with incidence ranging from 5 to 12% and rates of lower respiratory tract disease of 10 to 66%.[9,80,119,120] Notably, infection has been shown to have long-term complications in this population, including decreased lung function, bronchiolitis obliterans, and links to allograft rejection.[80–82] In one study, 82% of lung transplant patients with acute PIV infections who underwent transbronchial biopsy were shown to have acute allograft rejection and 32% subsequently developed bronchiolitis obliterans.[80]

Finally, increased risk of infection and persistent or severe disease has also been demonstrated in other special populations including children with other immunodeficiency syndromes such as severe combined immunodeficiency syndrome (SCIDS) who may have atypical presentations like parotitis or rapidly fatal giant cell pneumonia.[121] Though HPIV disease has not been well described in HIV populations, the correlation between lymphopenia and increased morbidity and mortality in leukemic and HSCT patients suggests that risk for severe disease likely increases with T cell depletion.

Other syndromes: Although HPIV infection is generally associated with respiratory tract illnesses, nonrespiratory complications of HPIV infection have been described in both adults and children. Parotitis may be an unusual manifestation of primary infection in children and has been described with HPIV1 and HPIV3 infections.[122,123] Infants with HPIV infection also may develop apnea and bradycardia, and infection in older children has been associated with exacerbation of nephrotic disease, hepatitis, and fatal rhabdomyolysis.[11,124–128] In adults, HPIV3 has been associated with myocarditis and pericarditis.[129]

Neurologic disease: Reports have described both acute and chronic neurological disease in children and adults associated with HPIV infections. Febrile seizures have been reported in young children, occurring with 62% of HPIV4 infections and 17% of PIV3 infections, and ventriculitis and encephalitis have been described in a few isolated cases.[124,130,131] Meningitis is a rare complication in both children and adults.[132–134] Interestingly, PIV infections have also been linked serologically to multiple sclerosis disease in adults, though evidence of true pathogenesis is lacking and MS has similarly been associated with other viral infections. Finally, PIV3 was isolated from the CSF of an adult with Guillain-Barre syndrome and other demyelinating syndromes have been described in adults with concurrent or recent PIV infections.[132,135]

Diagnosis

Laboratory diagnosis: Although the clinical syndrome of croup is commonly associated with HPIV1, most other

presentations of HPIV infection do not have unique features which allow viral infection to be diagnosed on clinical grounds alone. Thus, if the specific viral diagnosis is desired, laboratory testing is needed and can be accomplished by viral detection or host antibody response to infection.

Sample collection: Detection of virus whether by culture, fluorescent antibody assays, or molecular testing depends on the collection of an adequate sample. Several sample types are acceptable for testing and include nasopharyngeal swabs (NPS), combined nose and throat swabs (NTS), nasal washes, sputum, and bronchoalveolar lavage (BAL). The sample type will in part depend on the age and immune status of the patient and the severity and stage of the illness. Nasal washes which are commonly used in children are poorly tolerated by acutely ill older adults and NTSs are reasonable alternate specimens to collect.[103] If swabs are to be collected, it is recommended that flocked swabs be used in preference to cotton swabs due to enhanced yield.[136] The timing of sample collection during illness may also be important with upper airway samples being positive early in illness, whereas, later in illness, it may be more important to test lower airway secretions such as sputum and BAL fluid. In a study of hospitalized patients with documented parainfluenza illnesses, molecular testing of sputum added 33% to the diagnostic yield of collecting NTS alone.[137] BAL is generally reserved for severely ill or immunocompromised patients.

Viral culture: For many years, viral culture has been considered the gold standard for diagnosis. Viral isolation on cell culture depends on the development of cytopathic effect (CPE) or detection of hemadsorption (HAD) to the monolayers.[138,139] Confirmatory testing of CPE and HAD is accomplished through the use of viral-specific fluorescent-labeled monoclonal antibodies (Mab). Traditional viral culture demands an experienced clinical laboratory, and time to diagnosis limits clinical utility (5–14 days).[138] In an attempt to simplify the process, several commercially available mixed cell lines such as "R-Mix" have been used to successfully grow a variety of respiratory viruses including HPIV.[139] To accelerate the time to identification, the shell vial culture system utilizes low speed centrifugation of the inoculum on monolayers with Mab staining at 24 hours and expedites the time to diagnosis.[140]

Fluorescent antibody assays: Detection of viral antigens performed directly on clinical samples has been used since the 1970s as a rapid method for viral diagnosis.[139] Simple commercial colorimetric enzyme-linked immunoassays (EIAs) have been developed for RSV and influenza and perform reasonably well in children with primary infection where viral titers are high. No commercial rapid antigen test for HPIV is available. Direct detection of HPIV 1–3–specific immunofluorescent-labeled antibodies can be done with sensitivities of 63 to 95%; however, antibodies to HPIV-4 are generally not available.[11,139,141] Thus, when clinical resources are limited, testing of clinical samples by immunofluorescent assay (IFA) is a reasonable alternative.

Molecular assays: If available, molecular assays such as polymerase chain reaction (PCR) assays are the diagnostic test of choice for HPIV infection on the basis of optimal sensitivity, specificity, and rapidity of diagnosis.[21,142–144] Though PCR testing for the diagnosis of HPIV infection has been clearly shown to have superior sensitivity to viral culture and IFA testing, the clinical utility of PCR assays was at first limited by cost and the need for technical expertise to use for research and in tertiary-care facilities.[145–148] However, molecular testing has become more widely available with the development of commercial assays simplified for use in general clinical microbiology laboratories with rapid turnaround times of approximately 1 hour.[144,149,150] Initially developed as single-target assays, HPIV molecular assays are currently often imbedded in multiplex real-time PCR assays which test for respiratory viral pathogens including HPIV 1–4, with minimal loss of sensitivity for individual targets, though some variation in the sensitivity for different HPIV serotypes exists.[151,152] This variation may in part be due to the fact that though PCR primers are generally directed toward the HN gene, the specific sequence used varies by assay. Low viral loads can also be detected with PCR assays which may be important for early therapy and infection control in transplant populations.[146] Several sample types can be used for PCR testing including NPS, NTS, nasal washes, and BAL fluid. Sputum has been rarely used in molecular assays due to the viscous nature of the specimen, but new techniques have been described which allow the use of sputum samples for fully automated molecular assays.[137]

Serologic diagnosis: Serologic diagnosis is rarely used in clinical practice and is primarily a research tool. Complement fixation and EIA assays are available but require collection of convalescent sera to show fourfold or more rise in antibody titer and confirm acute infection. Cross-reactive immune responses to HPIV1 and 3 antigens make serotype-specific diagnosis of these infections by antibody response alone difficult.[153] Detection of HPIV-specific IgM has been described in children with HPIV infection, but commercial assays are not readily available.[154]

Treatment

Currently, there are no antiviral agents with proven efficacy for parainfluenza virus infection. Treatment of HPIV infection is generally symptomatic in healthy children and adults.[11]

Croup: Croup, commonly caused by HPIV1 and HPIV2 infection, presents with symptoms of a barking cough and stridor due to swelling and obstruction in the subglottic area of the trachea.[155] Corticosteroids are the primary treatment for croup and have been shown to be beneficial for mild and moderate to severe croup.[156,157] A fivefold reduction in rates of intubation has been noted in children with severe croup treated with corticosteroids compared with those not treated.[157] Among less ill children, corticosteroid treatment results in shorter emergency room visits, less frequent return medical visits, and improved sleep.[155] Corticosteroids may be administered by mouth or given intramuscularly in the form of dexamethasone or prednisolone and both have been shown to be superior to inhaled therapy with budesonide. Conventional dosing is a single dose of dexamethasone at 60 mg/kg, although lower doses have been proposed. The use of

nebulized epinephrine is associated with short-term relief of symptoms at 30 minutes, but treatment effects generally disappear after 2 hours.[158] This treatment may offer symptom relief while waiting for the anti-inflammatory activity of steroid therapy to take effect. Racemic and L-epinephrine are felt to have equivalent efficacy.[155] Despite a long history of using mist tents for croup, humidified air is not an effective treatment for croup. Heliox, which is a mixture of helium and oxygen has been proposed as a treatment for croup but is difficult to administer and does not offer significant benefits over conventional treatments.[155]

Antiviral agents: Presently there are no licensed antiviral agents for the treatment of HPIV infection. Data on the use of antiviral agents is primarily derived from animal studies, case reports, and small uncontrolled series in immunocompromised children and adults. The majority of treatment regimens utilize aerosolized or systemic ribavirin in combination with intravenous immunoglobulin (IVIG) and/or corticosteroids.[159] The nonrandomized nature of the these studies and differing routes of administration as well as the different underlying immune defects and type of HPIV infections treated (upper vs. lower tract disease) prohibit definitive conclusions for HPIV treatment. However, active research for new effective antiviral agents for HPIV is ongoing and several new agents show promise in vitro and in vivo.

Ribavirin: Ribavirin is a synthetic nucleoside analogue which has broad-spectrum in vitro and in vivo activity against many RNA and DNA viruses.[160] Aerosolized ribavirin is currently licensed for the treatment of severe RSV in young children and oral and intravenous ribavirin has been used for the treatment of other viral infections such as hepatitis C and Lassa fever.[161,162] Aerosolized ribavirin is generally well tolerated, although increased cough and bronchospasm may occur and systemic ribavirin can be associated with a reversible hemolytic anemia.[163,164] Unfortunately, most of the information regarding the utility of ribavirin comes from case reports or uncontrolled case series of patients with a variety of immunosuppressive conditions.[163,165–173] The bulk of the data are derived from persons with hematopoietic stem cell transplants (HSCT) which include solid-organ transplant recipients and primary immunodeficiencies. In children with SCIDS and HPIV infection, aerosolized ribavirin has been administered over long periods of time (3–10 months) without apparent toxicity.[164] Consensus indicates that ribavirin is not effective for HPIV pneumonia when given late in the course of illness, especially if respiratory failure has ensued.[7,9,174] Wendt and colleagues reported HPIV infection in 12 adults and 15 children undergoing HSCT with survival rates of 78% in those who received ribavirin as well as those who were not treated.[9] However, treatment was started after 11 days of illness on average. Nichols et al reported the treatment and outcomes of 253 HSCT patients with HPIV infection who were administered ribavirin within 48 hours of diagnosis and found no effect on 30-day mortality and the highest risk of death in patients with bacterial and fungal copathogens.[78,174] Finally, the largest series to date consisting of 544 HSCT recipients with HPIV infection treated at the Fred Hutchinson Cancer Center demonstrated that the use of inhaled ribavirin was significantly associated with reduced overall mortality but not mortality specifically from respiratory failure in multivariable analysis.[159] Moreover, in the subset with proven HPIV LRTI, there was no difference in mortality with ribavirin use.

Although the efficacy of ribavirin for the treatment of LRTI appears poor, early treatment to prevent progression to pneumonia remains an unanswered question with failures clearly documented.[163,168,170] In addition, the role of ribavirin to prevent long-term pulmonary sequelae has not been adequately studied.[7,80,172] In a small series of heart–lung transplant patients with HPIV infection, the use of a multidrug approach including IVIG, steroids, and ribavirin was associated with slower decline in lung function compared with historical controls.[175] In composite, the majority of studies do not provide compelling evidence that ribavirin provides significant benefit in the treatment of immunocompromised persons with HPIV infection and more effective treatments are critically needed.

DAS181: A novel approach which appears promising is a drug initially developed to treat influenza which acts on the host cell receptor for HPIV to prevent binding rather than exerting a direct effect on the virus.[176] The HN protein recognizes sialic acid containing glycolipids and glycoproteins on the host target cells and allows binding to occur.[177] DAS181 is an inhaled recombinant sialidase fusion protein that interferes with the initial binding of HN with the host cell sialic acid containing receptor.[13,178] Since sialic acid residues serve as the cellular receptors for both influenza and HPIV, DSA181 has been explored for HPIV antiviral activity.[179] This agent has been used under a compassionate use protocol to treat HPIV pneumonia in a lung transplant and an HSCT recipient with evidence of subjective and objective improvement.[178,180] Recently, DAS181 was used to treat two severely ill HSCT patients requiring mechanical ventilation and was successfully delivered via a nebulized formulation through the ventilator with significant decrease in viral load.[181] The drug was well tolerated with only a mild increase in serum alkaline phosphatase noted. Finally, four immunocompromised children infected with HPIV demonstrated clinical and radiographic improvement along with decreased viral load after treatment with DAS181.[182] The limited data available are encouraging and a phase 2 clinical trial in immunocompromised subjects with HPIV LRTI is ongoing (www.clinicaltrials.gov).

Other antiviral agents: Several other small molecules with in vitro activity against HPIV are in development.[183–185] The discovery of the 3D structure of the HPIV HN has allowed the design of inhibitors that fit into the binding site of the globular head to prevent binding and fusion of the virus.[13] Additional antiviral agents in development are HN inhibitors, BCX 2798 and BCX 2855, which bind to the catalytic binding site of HPIV and have been shown effective in the mouse HPIV model.[186,187]

Immunoglobulins: Immunoglobulin preparations contain neutralizing antibody to HPIV and may have anti-inflammatory effects. The administration of serum immunoglobulin (IVIG) has shown antiviral effects in the cotton rat model of

HPIV3 infection.[188] The combination of steroids with IVIG produced the most favorable results by reducing both viral titers and inflammation.[189,190] Although animal data are encouraging, data on the use of IVIG in immunosuppressed patients are conflicting. Case reports claim dramatic results with the use of IVIG, while larger observational studies find no benefit.[171,172,191,192] A recent report in which investigators noted no relationship between posttransplant levels of serum HPIV3-specific antibody and outcomes in HPIV3-infected HSCT recipients would suggest a limited role for IVIG in the treatment of established HPIV infection.[193]

Prevention

Vaccines: Currently, there is no licensed vaccine for the prevention of parainfluenza infection. Antibody to the two surface glycoproteins, F and HN are neutralizing and serum and nasal antibody to either protein protects against HPIV infection and ameliorates disease.[11,29,194] Thus, vaccines to boost serum and or mucosal antibody may offer benefit, yet several challenges to successful vaccine development remain. Cross protection between different HPIV serotypes is minimal or short lived, necessitating multiple or multivalent HPIV vaccines. Currently, most vaccine efforts are focused on HPIV3 which is the primary cause of severe disease and pneumonia in infants and in older adults. In young children, maternal antibody and the immature immune system are impediments to active immunization.[194] However, HPIV1- and HPIV2-associated croup infections occur at an older age and therefore the timing of vaccination could be delayed. Because of the disastrous results of the formalin-inactivated RSV vaccine trials performed in the 1960s during which enhanced disease with natural infection was observed, most HPIV vaccine research has avoided subunit vaccines.[195] Several approaches have included cold passaged attenuated live HPIV vaccines, bovine HPIV, and recombinant bovine/human HPIV vaccines.[194,196,197] As RSV and HPIV3 affect the same age group, recombinant vaccines that express both RSV and HPIV proteins are being explored.[196–199] Several candidate vaccines are now in phase I/II clinical trials in children.

Infection control: Transmission of HPIV is thought to be via large particle aerosols and fomites with self-inoculation.[11,21] Young children can excrete high quantities of virus which may be viable on porous surfaces for up to 10 hours.[200,201] Because small particle aerosols are not felt to be important mechanisms for transmission, droplet isolation is believed to be sufficient to prevent nosocomial spread in most health care settings.[21] However, prolonged shedding of low levels of HPIV has been documented in normal asymptomatic healthy adults as well as immunocompromised persons. Interestingly, two HPIV outbreaks occurred in healthy young adults 10 and 29 weeks after complete social isolation at the South Pole and were likely due to persistent low level shedding in some individuals.[202] Outbreaks of HPIV after HSCT have been reported in inpatient and outpatient settings and, despite aggressive infection control measures, have been difficult to control.[203,204] In several instances, outbreaks appeared to be centered in the outpatient facilities where waiting rooms were sometimes crowded and common infusion areas were utilized.[205] Because HPIV may cause prolonged asymptomatic infection, symptom-based infection control strategies which have been successful in curtailing RSV and influenza outbreaks may be less effective to prevent nosocomial spread of HPIV.[206] When HPIV outbreaks are detected in settings where immunocompromised patients are cared for, enhanced infection control measures are recommended including strict visitor and patient-to-patient contact limitation, cohorting, masking of personnel and visitors in contact with HPIV-infected patients, and frequent cleaning of environmental surfaces.[205] Screening of asymptomatic patients and staff may be indicated in difficult-to-control outbreaks.[206]

Conclusion

HPIVs cause a significant burden of disease in children and adults. A wide spectrum of illness including colds, croup, bronchiolitis, and pneumonia are attributed to these ubiquitous pathogens. The most severe disease is found among immunocompromised patients and treatment at present remains largely supportive. Several promising antiviral drugs are in development and are in early-stage clinical trials. Continued research for new vaccines and therapeutics is needed.

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